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FOREWORD

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for Clarence M. Page 7/27/95
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Introduction

During the period of this annual report (February 11, 1994 to May 30, 1995), the Epidemiology Research Center in Belize focused on further defining the epidemiology of malaria, Chagas' Disease and hepatitis B. Each of these diseases are proven or potential health problems in Belize. To address these problems, one study on malaria, three studies relating to hepatitis, and one study on Chagas' disease were undertaken or continued during this period.

1. "Use of a Questionnaire to study Malaria Incidence in Belize, C.A.", RO 87FK; Principal Investigator, Nancy Everett, RN, MPH

Malaria is becoming much more common in Belize with over 10,000 cases reported in 1994 in Belize, a country of approximately 200,000 people. Rural areas have much higher rates than urban areas. A project entitled "Use of a Questionnaire to study Malaria incidence in Belize, C.A." was conducted in the summer of 1994 among a rural group of Mayan Indians to determine possible reasons for high rates of malaria in their community. In this community, the rate of malaria was calculated at >400/1000 for 1993. This study was conducted by Nancy Everett, RN, MPH as part of her Masters in Public Health studies. Uniformed Services University provided funding for per diem and supplies for this study.

2. Etiologies of Hepatitis in Belize, G187BR-A1.

Principal Investigator CDR Joe P. Bryan, MD

This was a study of acute jaundice in Stann Creek district, an area where a number of cases of jaundice had been identified. Stann Creek district had

also been identified as an area with a high prevalence of hepatitis B in studies of the Belize Defence Force [1], pregnant women (unpublished data), and Health Care workers [2]. In addition, an outbreak investigation of hepatitis in workers at a banana farm in 1991 had indicated a high prevalence of exposure to hepatitis B and a sizable proportion of persons with IgM anti-HBc indicating acute hepatitis B [3]. For these reasons, an active surveillance system was set up to determine the cause of jaundice in persons over 2 years of age.

3. Seroprevalence of Hepatitis B Virus Among School-Aged Children in Belize, T087GC-01; Principal Investigator, Judith Chamberlin, PAC

Ms. Chamberlin, an MPH candidate at USUHS, led this study. Her travel and per diem were provided by USUHS. The objective of this study was to determine the prevalence of hepatitis B in school-aged children in Stann Creek district. Previous studies in adult members of the Belize Defense Force, health care workers and pregnant women had all indicated a high prevalence of hepatitis B in adults. With a high incidence of hepatitis B being documented in the etiology of hepatitis study, one of the agricultural companies agreed to donate funds for a hepatitis B vaccine program. In order to determine at what age the vaccine should be administered to protect the greatest number of children, the age at which children were becoming infected needed to be determined. Therefore, the prevalence of hepatitis B was studied among approximately 600 children at five schools in Stann Creek district.

4. Randomized Comparison of Three 5- μ g doses vs. Two 10- μ g Doses of Recombinant Hepatitis B Vaccine, G187BR-A1; Principal Investigator CDR Joe P. Bryan, MD

A hepatitis B vaccine trial was conducted among the Belize Defense Force. A previous hepatitis B vaccine trial among the Force had compared four different regimens of hepatitis B vaccine [4]. The results indicated that a reduced dose of vaccine, Recombivax HB (Merck Sharp and Dohme) administered in 3 doses of 5 μ g over 6 months provided an excellent seroconversion rate and high geometric mean titers of antibody. However, providing 3 doses of vaccine over 6 months was logistically difficult as recruits finish training and move on to field duty. Therefore, the present study was conducted among 2 groups of recruits in the Belize Defense Force who were in training for 14 weeks. Two regimens were compared: Recombivax 5 μ g IM at 0, 1, and 2 months and Recombivax 10 μ g IM at 0 and 2 months. If the regimens performed well, then immunization during a relatively brief period of training at a reduced price might be considered for other military personnel including those in the United States.

5. Prevalence of Chagas' Disease in Belize, G187BR-A1; Principal Investigator, Ruth Jaramillo, MT.

In the area of Chagas' Disease, one of the technologists at the Epidemiology Research Center continued work on the prevalence of antibody to *T. cruzi* among blood donors in Belize. She had previously studied sera from immigrant farm workers who were part of a hepatitis prevalence study, and members of the Belize Defence Force. A prevalence of approximately 4% was found in farm workers from other Central

American countries, but no reactive sera among approximately 500 members of the Belize Defence Force.

Manuscript Preparation and Scientific Presentations

In addition to the above projects, considerable investment of time was made in presenting other studies at meetings and preparing manuscripts. A study of hepatitis B among health care workers was presented by Linda Reyes, one of the medical technicians at the American Society for Tropical Medicine and Hygiene meeting in November 1994. The study was also presented at the Caribbean Commonwealth Medical Research Council Meeting in Barbados by Shilpa Hakre in April 1995. The manuscript has been accepted for publication [2]. Some preliminary data on the Chagas' study was presented at the Annual Meeting of the American Society for Microbiology meeting in Las Vegas and at the Tropical Medicine Meeting in Cincinnati [5]. A manuscript of this work is being revised.

The study comparing the four regimens of hepatitis B vaccine has been accepted for publication in Vaccine [4].

Entomologic Studies

The entomology group at USUHS used the Epidemiology Research Center as a base from which they performed several studies. In addition to entomologists at USUHS, an entomology team from Ft. Detrick led by Major Russ Coleman performed some studies in Belize during the summer of 1994 using the ERC as their base.

Medical Student Rotations

During this period, three USUHS medical students spent time in Belize. The students paid their own travel and living expenses. They worked at the Belize City Hospital, the Belize Defense Force, British Forces Belize, and at the District Hospital in Dangriga, Stann Creek District. One student helped with the school based survey for hepatitis and another helped find and examine cases of acute jaundice in the study of acute hepatitis.

Pakistan Project

In addition to work in Belize, work continues on hepatitis E in Pakistan. This work is being done with collaborators at the Walter Reed Army Institute of Research and at the National Institutes of Health. A complete description with serologic results of an epidemic of hepatitis E in Sargodha, Pakistan was published in the Journal of Infectious Diseases [6]. A manuscript of the serologic results and pattern of Hepatitis E excretion in feces from an epidemic of hepatitis E which occurred in Abbottabad has been prepared. A small portion of this material was presented at the American Society for Tropical Medicine meeting in 1994 and at the Triennial Symposium on Hepatitis and Liver Disease held in Tokyo, Japan, 1993.

METHODS:

1. Use of a Questionnaire to study Malaria Incidence in Belize, C.A.

Nancy Everett, as part of her MPH degree, elected to study social correlates of malaria among Mayan Indians in the southern part of Belize. A village was selected based on its high rate of reported malaria and

accessibility, being about a 4 hour drive south of Belize City. A questionnaire was developed during the spring of 1994. The USUHS Human Use Review Committee determined the study to be exempt from review. In July 1994, Nancy Everett tested a pilot questionnaire in Red Bank for a couple of days, then revised it. With the help of ERC technicians, Ruth Jaramillo during one week and Shilpa Hakre another, the questionnaire was administered to a large portion of the village. The main objective was to determine if malaria was being transmitted in the work place or in the village itself.

A map of the Red Bank area was constructed indicating the dwellings. Households were selected for questionnaires by randomly selecting the first house, then taking every third house in the housing section thereafter. Terms of confidentiality were explained and informed consent was obtained from the head of the household. Interviews were usually attempted in the afternoons when men had returned from the farms.

The questionnaire was comprised of 5 sections: housing, mobility, mosquito biting, personal protective measure, and methods of diagnosis and treatment of malaria. For the Mayan speaking persons, English was the language used, though the question was frequently discussed among respondents in a Mayan language before answering in English. In one section of Red Bank inhabited by Spanish speaking persons, that language was used.

A definition of malaria was any illness with repeated fever, chills, night sweats and headache. If no symptoms of this nature had occurred in the past 12 months, no more questions were asked. Attempts were made to verify the results of malaria smears of persons who reported malaria

from the malaria officials in Dangriga, the district capital about half way between Belize City and Red Bank.

2. Etiologies of Hepatitis in Belize

This was a longitudinal study of acute jaundice in Stann Creek District. After approval by the Human Use Review Committee in Belize and then by the HURC at USUHS, a study of causes of acute jaundice was inaugurated in March 1994 and continued through May, 1995. The main purpose was to determine the etiologies of jaundice and document the incidence.

Every six weeks, a team from the ERC, usually accompanied by CDR Joe Bryan or one of the USUHS medical students, traveled to Stann Creek district. Surveillance for cases occurred in 5 ways: Persons who presented to the Stann Creek District hospital were attended by Dr. Mohan Kishore, a graduate of the USUHS MPH program and who was then the district medical officer in Stann Creek; persons with an elevated bilirubin in the lab at the Stann Creek district hospital; surveillance for persons with jaundice who presented to Dr. Hailu in Independence in the far south of the district; active surveillance by the nurses who conducted mobile clinics in the rural areas of Stann Creek district, and active case finding by the ERC team as we went to the banana packing sheds and checked with the mayors or other responsible persons in various villages.

Questionnaires were administered by one of the ERC technologists or physicians. The lab samples were sent either directly to the ERC with regular laboratory specimens by plane for Dangriga or carried by ERC staff during case finding visits. Each visit by ERC staff required 3 days, was fraught with dangers from dusty dirt roads, banana and orange trucks, robbers, and mechanical break downs.

Laboratory testing was performed on blood from cases for chemistries and a complete blood count, a malaria smear and IgM anti-HBc, IgM anti-HAV and HBsAg. If these hepatitis serologies were negative, sera were tested for total anti-HBc and anti-HAV. Household contacts of cases were tested for total anti-HBc (HBsAg if anti-HBc was present) and anti-HAV. Results of the questionnaire, the physical examination, and the laboratory reports were entered into a computer.

3. Seroprevalence of Hepatitis B Virus Among School-Aged Children in Belize

After approval by the Belize Human Use Review Committee and the HURC at USUHS, a study to determine the rates of hepatitis B exposure in various groups of school children in Stann Creek district was begun. Preliminary work was done in January 1995, when we informed and sought approval for the project from school boards. In February of 1995, Dr. Bryan and ERC staff visited the principals of 5 schools (2 high schools and 3 primary schools). Educational materials developed by Judith Chamberlin were distributed to the principals. A date to meet with the parents of the schools was set for March. Letters about the project were sent home with students.

At the meeting with the parents in March, Judy Chamberlin and CDR Bryan explained the reasons for the study, the procedure for informed consent, blood drawing, value of the study to individuals and the community, and then answered questions. On a subsequent day at each school, children with signed consent forms underwent venipuncture for 7 ml of blood. Demographic data on the children were obtained from a questionnaire completed by the parents.

Blood samples were examined for malaria parasites, spun hematocrit, and anti-HBc and HBsAg. The results of this testing which was all done in Belize at the ERC or Central Medical Laboratory were entered into a computer and a personal report was generated and distributed for each student approximately 6 weeks later.

4. Randomized Comparison of Three 5- μ g doses vs. Two 10- μ g Doses of Recombinant Hepatitis B Vaccine

After approval by the Human Use Review Committees in Belize and at USUHS, members of the Belize Defense Force who were in the recruit intakes in the summer of 1994 or January 1995, were invited to participate. Some others who had been in other recent recruit intakes were also invited. After signing a consent form, 5 ml of blood was obtained at the time of blood collection for other laboratory testing routinely performed on all members of the Belize Defense Force. Testing for anti-HBc was performed. Sera which were reactive were repeated in duplicate. Those repeatedly reactive were tested for HBsAg.

Members who tested negative for anti-HBc were randomly assigned to one of two groups. Recombivax HB 5 μ g in 0.5 ml was administered at 0, 1, and 2 to 3 months to one group while Recombivax HB 10 μ g in 1.0 ml was administered IM at 0 and 2 to 3 months to the other.

Sera were collected at the time of the final dose and 6-12 weeks after the final dose.

Sera will be assayed for quantitative anti-HBs. All sera will again be tested for anti-HBc to detect any intercurrent infections.

5. Prevalence of Chagas' Disease in Belize

Samples of blood from persons who donated blood through the Belize blood bank were tested for antibody to *T. cruzi* using an ELISA test kit. The samples were collected in an anonymous unlinked manner. Results of testing were not released to patients or laboratory personnel. This was a continuation of a study started in the fall of 1993.

6. Samples of serum and feces collected from men in Abbottabad, Pakistan who were involved in an outbreak of hepatitis in 1988 after accidental contamination of the water supply were studied. Sera were studied by ELISA for anti-hepatitis E antibodies at NIH (Dr. Robert Purcell/Sergei Tsarev) and feces were studied by affinity capture PCR for HEV at WRAIR (Drs. Longer and Ticehurst and staff).

RESULTS:

1. Malaria survey

Red Bank is a village of approximately 300 persons. Information from the malaria control report indicated Red bank had the highest incidence of reported malaria in Stann Creek district during 1993 with an incidence of almost 400/1000. Other Mayan villages in the area had rates of about 200/1000 in Santa Rosa, 180/1000 in Maya Mopan with slightly lower rates reported from the Spanish speaking areas of Cowpen and Trio Bladen (approximately 150/1000 each). All these villages are in the banana growing areas and are close to a river where bathing and washing are conducted. Rates in the coastal towns of Hopkins, Dangriga and Independence were much lower, < 25/1000.

Thirty-eight families out of approximately 60 households in the village of Red Bank were interviewed. This represented the experience of 197 individuals. The male/female ratio of those responding was equal. Many persons indicated they had malaria in the past 12 months: Approximately 56% of 78 persons 0-9 years of age were said to have had malaria, 37% of 43 persons 10-19 years; 61% of 57 persons 20-44 years; 50% of 14 persons 45-64 years of age; and 40% of 5 persons over 65 years of age. A similar proportion of male (46%) and female (54%) indicated they had malaria in the past year.

The average size of each family was 5 persons. The average length of time residing in Red Bank was 6.3 years. The village had been relocated by the government some time in the past to the present location. Most houses were thatch, though some had cement or wood construction; none had screened windows or doors. All participants reported mosquitoes biting indoors between the hours of 6 PM and 5 AM. Most (72%) reported more mosquito biting during the rainy season.

Work patterns indicated that the men worked in their milpas (farms) from dawn to early to mid afternoon or worked on the nearby banana plantations while the women stayed in the village. Some women worked in the banana packing sheds nearby.

Travel patterns indicated that only 5% of respondents had traveled out of Belize in the past year indicating malaria transmission must be occurring in Belize. Bathing occurred in nearby streams or rivers, usually during the morning (women) or afternoon (men). Almost all spent evenings indoors; only 3 persons indicated spending nights away from the village.

Personal protective measures were not used routinely. Bed nets were not routinely used. Most people slept in hammocks or on flat beds covered with cardboard.

Of persons indicating a history of malaria in the past year, most (80%) stated that a malaria smear had been obtained at the time of the illness, though few remembered receiving results. Attempts to confirm results of malaria slide testing with the malaria control personnel in Dangriga were not very successful. Difficulty was experienced in sorting through the results in a log book compared with names as given in the questionnaire.

Medication usage for malaria generally fell into 2 categories. About half (46%) indicated they took 5 or more days of chloroquine (the government approved regimen at that time was 5 days of choroquine and primaquine given once daily) while 46% indicated they took medicine for only one day.

2. Etiologies of Acute Hepatitis in Belize

Most cases of acute jaundice were found in the southern part of Stann Creek district. The most common profile was that of a young single Spanish speaking male 18-39 years of age living near the banana plantation. However, a number of married men and women were also found to have jaundice. Another common profile was that of young men or women of Mayan descent living in the villages. Final data remain in Belize. However, analysis of data through February 1995 indicated approximately 70 cases of clinical jaundice had been entered into the study. Laboratory testing was complete on approximately 50. Of these, acute hepatitis B was the etiology in approximately 39, acute hepatitis A

in 4 (all children), and malaria parasites, including *P. falciparum* were observed in several others.

Among approximately 50 contacts of cases, most had been exposed to hepatitis B, and a number carried hepatitis B surface antigen.

3. Seroprevalence of Hepatitis B Virus Among School-Aged Children in Belize

A seroprevalence study of hepatitis B markers was conducted among school-aged children in that district to provide information for planning a hepatitis B vaccine program. After informed parental consent, 587 students, aged 4-22 (mean 13 years), from five schools were tested for antibody to hepatitis B core antigen (anti-HBc) and hepatitis B surface antigen (HBsAg). The overall prevalence of hepatitis markers was high: 43.3% had anti-HBc and 7.7% had HBsAg. Anti-HBc was more common in males than females (52% vs. 36.5%; p<.05). There was also marked variation between ethnic groups.

	Mayan (n=133)	Mestizo (n=92)	Garifuna (n=173)	Creole (n=141)	Other (n=48)
Anti-HBc +	76%	50%	37%	25%	19%
HBsAg +	9%	11%	9%	4%	2%

At the two rural primary schools attended mainly by Mayan and Mestizo children, >65% of children ≤ 8 years had anti-HBc, with no increase with age. In contrast, at the urban primary school attended mainly by Garifuna and Creole children, only 9% of children ≤ 8 years had anti-HBc, and seropositivity increased with age (p <.05, by chi-square test for trend). Anti-HBc was found in 42% and 36% of students at the two high schools.

Malaria parasites were observed in 5 students; 4 in the Mayan community and one in the Spanish speaking community. Spun hematocrits indicated the lowest hematocrits among the Mayan children, with intermediate levels in the Spanish community. Few cases of anemia (<35%) were observed in the coastal towns.

4. Randomized Comparison of Three 5- μ g doses vs. Two 10- μ g Doses of Recombinant Hepatitis B Vaccine

During the screening phase, 265 members of the BDF were tested for anti-HBc. Of these 65 (25%) had antibody to hepatitis B core antibody. Of the 200 eligible persons (without anti-HBc), 172 received the first dose of vaccine and 154 received all doses. No results of antibody testing are available at this time.

5. Prevalence of Chagas' Disease in Belize

Results of screening blood bank samples collected in 1993 and 1994 indicated that 8/920 (0.08%) of samples were repeatedly reactive for antibody to *T. cruzi*. Several of these were among persons not born in Belize.

6. Hepatitis E in Pakistan

An outbreak of hepatitis which occurred among men in an academic community in Abbottabad, Pakistan, was traced to contamination of a water supply. Of 109 men hospitalized with a clinical diagnosis of hepatitis, 104 (95%) were found to have serologic evidence of acute hepatitis E. Both IgM and IgG anti-HEV were present in 92% of cases on admission. Among 44 men from whom three serum specimens were

obtained over 4 months, IgG anti-HEV geometric mean titers (GMT) decreased from 1534 on admission to 651 at 4 months. IgM anti-HEV was detected in 40 (91%) of 44 men at a GMT of 525 during acute disease but was observed in only 8 (18%) four months later. Affinity-capture PCR (AC/PCR) detected HEV in serially-collected feces from 18 of 19 men. The intensity of viral excretion was greatest during the first week of symptoms (present in 79% of patients) but persisted intermittently for up to 4 weeks after the onset of jaundice. PCR studies of single fecal specimens from 41 patients detected HEV in 13 (32%). This study identifies HEV as the etiologic agent of this outbreak, elucidates the pattern of anti-HEV in patients, and demonstrates the pattern of HEV excretion in feces as measured by AC/PCR .

Conclusions

1. Malaria questionnaire study

This study gives considerable insight into why such a high rate of malaria is experienced in this community, though many questions remain. One weakness of the study is that verbal reports of malaria-like illness were not confirmed by blood smear results. Never-the-less, that 53% of villagers indicated a malaria-like illness seemed consistent with the slide confirmed rate of 430/1000 of persons in the village in 1993. The study did suggest that malaria transmission was occurring in the village since villagers were not traveling outside Belize or even to other areas. Second, since the rates of malaria as reported on the questionnaire were similar among young women who usually stayed in the village compared with men who work on farms, transmission is likely occurring in the village.

Personal protection from malaria was lacking since mosquito repellent was seldom used, bed nets were not often used, and doors and windows were not mosquito proofed. No spraying of insecticide in the village or in the homes had been conducted in some time. Implementation of these measures could reduce exposure to mosquitoes or reduce the number of mosquitoes in homes or in the village.

Another area of concern was the lack of laboratory diagnoses of malaria. While slides were made by volunteer coordinators, results of the testing were apparently not available in a timely manner. This is in part due to the difficult logistics of sending slides 2-3 hours to Dangriga and returning the results in a timely manner. Only one telephone exists in Red Bank, a community phone, so telephoning results might be one solution to returning results in a timely manner.

Another area where improvement could be made is that of therapy. While about half of the people took malaria medicines as prescribed by the malaria control program (one chloroquine daily for 5 days), another half took only one tablet of chloroquine. This may be because of lack of training on the part of some volunteer collaborators, or lack of understanding by patients. This practice may result in some improvement in symptoms, but is not curative and could result in the development of resistance.

Another possibility is that since persons are not routinely taking primaquine, some malaria may be resulting from relapses of *P. vivax*, the predominant (95%) kind of malaria in the area.

Therefore, the study indicated several areas in which work needs to be done to improve the malaria situation including improved housing, sleeping under bednets, use of repellent, better training of volunteer

collaborators, improved reporting of malaria slide results within 1-2 days, mosquito reduction measures, and possibly chemoprophylaxis with choroquine during periods of high mosquito densities. Other areas of work might include night biting studies in various locations.

2. Etiologies of Acute Hepatitis in Belize

This study indicates that the major cause of jaundice in this area is hepatitis B. The study defines those with the highest incidence as immigrant farm workers on the banana plantation and Mayan Indians, many of whom also work on the plantations. The study raises a number of questions and has major implications. Questions are raised such as why hepatitis B is so common, how is it being transmitted, and how can it be stopped. Probably the only way to stop hepatitis B in this area is through a hepatitis B vaccine program. If a vaccine program is instituted, who will be vaccinated. Would it be most effective to immunize adults, children or infants? To answer this question, the study on the prevalence of hepatitis B in school age children was conducted.

Fortunately, representatives from the multinational company that raises banana has agreed to supply some funds for a hepatitis B vaccine program. This study, along with the study conducted in 1991 at Cowpen, was very influential in encouraging the company to support this program. Presently, a committee in Belize is preparing plans to implement a hepatitis B vaccine program in Stann Creek District among all infants in this district, as well as the children who participated in the school based survey who tested negative for hepatitis B. CDR Bryan has been serving on this committee. Therefore, this is a study that has defined who, what, and

where disease is occurring and has resulted in the implementation of control measures.

3. Seroprevalence of Hepatitis B Virus Among School-Aged Children in Belize

Among school-aged children in Stann Creek District, the prevalence of HBV infection varies by location, ethnicity and age. The prevalence of hepatitis B among children in the towns was much lower than among children in the rural Mayan community or Spanish speaking community. Because most children in the rural areas are exposed to hepatitis B before entering school, immunization against HBV should be integrated into the routine infant immunization program. A school based hepatitis B vaccine could be integrated into the primary grades of the schools in town. More work needs to be done to define the etiologies of anemia in the rural areas. Malaria was documented in children in the village where the questionnaire study was performed.

4. Randomized Comparison of Three 5- μ g doses vs. Two 10- μ g Doses of Recombinant Hepatitis B Vaccine

No discussion of the feasibility of using this abbreviated schedule with lower doses is possible at this time because no results of anti-HBs titers on persons in this study are available.

5. Prevalence of Chagas' Disease in Belize

The finding in blood bank samples of a prevalence of 0.08% suggests that exposure to *T. cruzi* in blood donors is not very common. Among members of the Belize Defense Force, none of approximately 500 members

tested with this same method were reactive. However, among immigrant workers in the banana plantation, a prevalence of approximately 4% was found. This suggests that many people living in Belize are not exposed to *T. cruzi*; however, immigrant workers from Honduras, Guatemala, and El Salvador appear to have some exposure. Whether this is occurring in the rural area of Belize where they are presently working, or whether they were infected in another country is unknown.

An entomological study for Triatome vectors of Chagas' disease in the area of the rural workers would be worth while. In addition, a study of the incidence of new antibody among workers in this area should be considered.

For the blood bank, a low rate of antibody has been documented. Perhaps a questionnaire could be used to determine those who came from rural areas of other Central American countries where they may have been exposed.

6. Hepatitis E in Pakistan

This study identifies HEV as the etiologic agent of this outbreak, elucidates the pattern of anti-HEV in patients, and demonstrates the pattern of HEV excretion in feces as measured by AC/PCR. It further demonstrates that anti-HEV prevents re-infection with HEV. The pattern of HEV in feces has implications for infection control with regard to the potential infectious nature of feces from cases. The demonstration that protection is provided by anti-HEV gives hope that a vaccine against hepatitis E may someday be a reality.

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